Intestinal Microbiota in Personalized Medicine

Subjects: Medical Laboratory Technology

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The microbiota is like a unique personalized “mold” for each person; it differs quantitatively and qualitatively for the microorganisms it contains together with the relationship between them, and it changes over time and under the influence of many factors.

microbiota intestinal microbiota oral microbiota immune system and dysbiosis probiotics

1. Biomolecular Mechanisms of Intestinal Dysbiosis

The intestinal microbiota and the host coexist in harmony (eubiosis) and from this there is mutual benefit. The host provides the space and suitable conditions (nutrients, presence of O_2 or microaerophilia, temperature, and pH) for the growth of the microbiota, thus participating in the metabolic pathways of the host, producing useful substances that cannot be produced by the host, or inducing the immune response of the host to various infections. Therefore, the metabolism and fermentation of many nondigestible food components, such as fibers, some lipids and proteins, bile acids, cholesterol and so on, is one of the most important functions of the microbiota in the large intestine (7–10% of the host’s daily energy requirement) [1][2]. In this way the bacteria provide energy but also produce short-chain fatty acids (butyric acid and propionic acid), which are an additional source of energy for the host. These acids have the utility of: (a) supplying energy to colon cells and bacteria, (b) activating the mechanisms that promote the integrity of the tissues of the area, (c) influencing the immune system and immunization, (d) influencing the onset of metabolic diseases (obesity, osteoarthritis, and diabetes diabetes), (e) having anti-inflammatory action, (f) having anti-apoptotic action, (g) regulating lipogenesis, (h) regulating appetite hormones and pH, and (i) contributing to nutrient absorption. Some bacterial species can synthesize amino acids and vitamins (such as K, B12, folic acid, thiamine, biotin and so on). The *Bacillus thetaiotaomicron* is responsible for the breakdown of polysaccharides that become indigestible in the large intestine through the presence of various enzymes such as glycosides hydrolases and lyases of polysaccharides that break down pectin, arabinose and so on. The “friendly” bacterial species, such as *Lactobacillus spp.* and *Bifidobacteria spp.*, lack the proinflammatory external lipopolysaccharide (LPS) chains, which are anchored to the cell walls of pathogenic bacteria such as *E. coli* and the genus *Salmonella* [3][4][5][6][7][8]. Symbiotic bacteria of the microbiota secrete antimicrobials such as bacteriocins and hydrogen peroxide, thus inhibiting the growth of other pathogenic bacteria. There is also competition for both the location of each other, and the availability of present nutrients in the lumen. The microbiota regulate the development and function of the innate and acquired immune systems. In the circumstances of eubiosis, constant stimulation of the immune system by the gut microbiota leads to a state of “low normal inflammation”, which is a direct and effective defense mechanism against pathogens. Furthermore, the flora

competes with its protective role, metabolizing the nutrients necessary for the survival of pathogens and producing molecules that inhibit the growth of these bacteria [3][4][9]. Therefore, the function of the intestinal microbiota in terms of the defense of the organism is, on the one hand, to influence the intestinal immune mechanism and, on the other hand, to prevent the possible invasion of pathogens by directly affecting them and/or by "activating" the immune system of the host [10]. In fact, through natural immunity, the molecular patterns associated with characteristic pathogens (PAMP) are identified on the microorganisms, thus selecting potentially pathogenic from nonpathogenic microbes. More specifically, natural immune cells have specific PRRs (pattern recognition receptors) that bind to PAMPs. PRRs are involved in the activation of acquired immunity and the release of cytokines, for example, the Toll-like receptors (TLRs), which are found in macrophages, neutrophils, dendritic cells, and the epithelial cells of the intestinal mucus [11]. PAMPs recognized by PRRs are bacterial carbohydrates (such as lipopolysaccharide-LPS and mannose), nucleic acids (viral DNA or RNA), bacterial peptides (such as flagellin), peptidoglycans, and fungal glucans from liposuction. However, since all of these are present and are also found in symbiotic microbes, they are referred to by the term MAMP (molecular models associated with microbes) [11][12]. Through the recognition of MAMPs, symbiotic microbes change the expression of TLRs in natural immunity cells and trigger the activation of the NF-κβ pathway which stimulates the production of cytokines and ultimately results in the activation of T lymphocytes, for example, acquired immunity. As it was highlighted, gut germs can change the quantity of mucus produced by the cells of the intestinal mucosa and thus play a protective role in conditions of eubiosis which will activate the body's defenses, protecting it from pathogens [3][13]. Hence, commensal bacteria prove necessary in eubiosis for the aid of regular digestion, for the normal development/function of the immune system (intestinal, mucosal, and systemic), to lower the pH with short-chain fatty acids (SCFAs), to secrete antitoxin proteins (bacteriocins) against toxin-producing bacteria, and to exert an important defense against colonization by non-commensal microorganisms with the regulation of intestinal mucus. Therefore, there are host–microbe local interactions involving various organs, creating the gut axes [13][14][15][16][17][18][19].

Thus, under suitable conditions, a long-term symbiosis with many benefits for the host's health may exist. However, when for some reason the conditions change, the composition of the microbiota also changes, resulting in pathological conditions, infections, inflammations, and various psychosomatic diseases. This condition is called dysbiosis. A dysbiotic state allows the settlement of non-friendly, and therefore pathogenic, bacteria in place of the resident "friendly" commensal bacteria.

The environmental factors mentioned previously, specifically unhealthy lifestyle choices (such as low or exhausting physical activity levels, psychogenic stress, or smoking), exposure to toxic substances (such as industrial chemicals, heavy metals, or abuse of antibiotics), and "bad" diets (overconsumption of sugar, alcohol, caffeine, or spicy foods, low-fiber diet and so on), activate in combination with the genetic predisposition of the host (idiosyncrasy) to become an abnormal irregulate function of the host's immune system. This condition can be cause a chronic inflammation of the intestinal mucosa, which in turn is a potential risk factor for idiopathic inflammatory bowel disease (IBD) and other severe chronic diseases [20][21][22][23]. In pathological conditions of the host, such as in the case of Crohn's disease, the disease is mainly associated with cytokines of T1 helper cells (factor TNF-α, interleukin-12: IL-12, and interferon-γ or IFN-γ). When mucosal injury occurs, epithelial cells are transferred to the site of mucosal injury for healing and rehabilitation. According to recent scientific data, an

unexpected immune response to acute injury in Crohn's disease patients is indicated. People suffering from this IBD show low neutrophil accumulation and lower IL-8 and IL-1β production. There is also talk of damage (defect) in the immunoregulation, which implies the perpetuation (worsening) of inflammation. Crohn's disease, as in ulcerative colitis, also activates CD4 helper T cells which are responsible for the secretion of proinflammatory cytokines. In contrast with the morbid condition of the host where their activation is observed, CD4 helper T cells and epithelial cells in normal state activate CD8+ suppressor cells [3]. Patients perceive endurance in T-cell apoptosis which is attributed to IL-6. The macrophages and monocytes may release sIL-6R (a soluble interfering receptor) that binds to IL-6, pushes gp130 to the cell surface, and induces anti-apoptotic gene expressions. Even the cells of the mucosa of patients tend to be associated with leukocytes compared to healthy individuals, which indicates that the non-involved cells in the immune response take part in the pro-inflammatory formation of chronic inflammation [20][24][25].

Genetic factors are thought to have a direct effect on the composition of the gut microbiota leading to the condition of dysbiosis. The epithelial cells of the intestinal mucosa are the first line of defense against pathogenic microbes. Although these cells are in constant relationships with germs and their products (despite being pro-inflammatory agents for other cell types), they do not react with a defense response. Hence these cells in the intestinal environment provide protection to the host from an inflammatory response against the microbiota. Therefore, the role of intestinal cells is the ability to recognize pathogens [3][25] and only infection with these pathogens will induce a proinflammatory response. It has been found that the NOD2/CARD15 gene participates in this intracellular discrimination system of intestinal epithelial cells. It is also characterized as a cytoplasmic protein, the expression of which is limited to monocytes/macrophages. Furthermore, it can be expressed in other cell types or caused after treatment with proinflammatory agents (IFNγ or TNFα) [25][26]. Its role is attributed to the activation of the NFκβ transcription factor pathway, the main regulator of proinflammatory cytokines (TNFα and IL1b) that induce inflammation.

Mutations in the NOD2/CARD15 gene inhibit the pathogenic or nonpathogenic microbial identification mechanism, disrupting the normal cytokine inhibition mechanism with consequent dysbiosis of the microbiota leading to significant inflammation of the intestinal mucosa [27][28]. Gut microbiota dysbiosis was noted in mice in which the NOD2/CARD15 gene is not expressed. Indeed, levels of the phyla *Bacteroidetes* and *Bacillota* (such as *Bacilli* spp.), were particularly high in mice that had mutations in this gene versus those that did not. Furthermore, after colonization of the intestine of mice with *Helicobacter hepaticus* the fecal microbiota in the following days of those without mutations showed a greater ability to eliminate this pathogen bacterium; in contract to those that had mutations. Similarly, it also occurs with *H. pylori* for its mutagenic and carcinogenic power in the gastric mucosa [29][30][31][32]. The NOD2 gene contributes to the identification of microorganisms with a harmful effect on the intestinal mucosa, providing host protection from their colonization. It was observed that patients with Crohn's disease or ulcerative colitis and mutations in the NOD2 gene showed low populations in intestinal biopsies of the genera *Clostridium* XIVa and IV with high presence of *Actinomycetota* and *Pseudomonadota* phyla [33]. It was also noted that individuals with IBD and NOD2 gene mutation present a dysbiosis of the intestinal microbiota with a balanced/disturbed immune system with a high presence of *Enterobacteriaceae* [34]. The ATG16L1 gene regulates the breakdown of proteins in the lysosome, the production of cytokines, and cell homeostasis. A correlation of its

mutations with intestinal dysbiosis was observed. Indeed, in individuals with Crohn's disease (in which the disease was in recession) who had a mutation in the ATG16L1 gene, intense activity of the GRP78 and peIF2α markers was noted. These markers detect the endoplasmic stress of the Paneth cellular network [32]. It has also been noted that if there is an important stress condition, individuals are more likely to develop idiopathic inflammatory disease in the small intestine and may have surgical complications, such as Crohn's fistulas [35]. Additionally, increased stress indices have shown elevated levels of *Escherichia coli* in intestinal biopsies. Finally, high concentrations of the species *Bacteroides*, *Fusobacteria*, and *E. coli* with low presence of *Lachnospiraceae* (family of bacteria belonging to the order *Clostridiales*) in tissues with inflammation, were observed in patients with IBD with a defect of this gene. An important factor in intestinal microbiota dysbiosis is bacterial translocation, defined as the transport of germs through the intestinal mucosa to sterile areas (mesenteric lymph nodes and abdominal organs) [36][37]. This translocation is observed in patients with Crohn's disease and ulcerative colitis. Bacterial translocation therefore includes transport through the vulnerable intestinal mucosa of antigens and endotoxins into the systemic circulation, thus inducing the formation of inflammation and damage to various organs. In host conditions, such as inflammatory bowel disease, a hostile environment is formed in the gut with a modified microflora composition; bacterial translocation in these diseases is attributed to either lesions observed in the gut mucosa or mutations in the CARD15 and ATG16L1 genes [36][37][38][39][40].

2. The Importance of Gut Microbiota Testing to Reveal Host's Dysbiosis

As noted, the qualitative (type of bacteria) and quantitative (other changes in the number of species) variations of the gut microbiota relate to the state of well-being of the organism. Unveiling its dysbiotic composition allows in advance or in time to preserve it or correct it to reach eubiosis. Thus, it could be avoided, cure, or reduce the risk of some pathologies, as mentioned. With the presence of advanced molecular techniques for highly sophisticated analyses, it is possible to characterize the components and microbial functionality of the intestinal microbiota with more precision. The analysis of the intestinal microbiota is performed using a special kit for taking a fecal sample (it can be stored for up to 4 weeks at room temperature). The genetic patrimony expressed from the intestinal microbiota, results to be more rich respect of other individual niche, and therefore is indispensable in the homeostasis of overall health. Thus, it was necessary to investigate the composition of the intestinal microbiota, to check their state of well-being; it could therefore, in cases of dysbiosis, indicate a targeted therapy [41][42].

It is possible to analyze different parameters even if the aspects most considered and analyzed are the biodiversity index (alpha diversity) and the possible degree of dysbiosis on the composition of the microbiota (a eubiotic microbiota is characterized by a high level of taxonomic diversification). In particular, from the sample, it is possible to obtain: (a) descriptive analysis of the relative abundance of the various bacterial species, (b) the degree of metabolic efficiency, (c) an evaluation of the presence of potentially pathogenic bacterial groups (such as *C. difficile, C. perfringens, Salmonella, Klebsiella, Enterococcus faecalis and so on*), and (d) an evaluation of the physiological functions expressed in "indices" calculated on the basis of the relative overpopulation of the species involved in that function [43].

The sample is then analyzed through massive sequencing (next generation sequencing) so it is obtained through a bio-computing processing and statistical analysis of the data for the identification of all the bacterial components of the microbiota in question. Then, the analysis is performed from a sample of 1–2 g of feces, from which the DNA of the bacteria is extracted in the laboratory and then purified and amplified by NGS. Therefore, based on the quantitative and qualitative variations obtained from the sample, a complete and usable picture of how this can impact the physiology of the host is returned by applying a method of functional interpretation [44]. Then the examination of the microbiota detects the “fingerprint” of the bacterial component and analyses its overall state of balance and functionality. Based on the results obtained, it will in fact be possible, if necessary, to adopt the right corrective strategies, such as changes to nutrition or lifestyle, integration with probiotics and/or prebiotics and so on. As mentioned, the test is designed to utilize the rRNA 16S gene as target and amplification primers for PCR and probe for hydrolysis, which enhances the specificity of the dose. Each qPCR DNA microbial DNA sampler analyzes two samples simultaneously [43][45]. The qPCR microbial DNA metabolic distillation matrix is a search tool used for screening or regulating profiling and test strips of test samples, associates, and obesity, type 2 diabetes mellitus, metabolic syndrome, and other diseases. Identification is the determination of the presence of microbes in the sample that enable the excision of a control of the model during analysis [46]. Positive indices that are obtained are important for maintaining the health of the host, so they are obtained when the intestinal microbiota has the characteristics necessary to efficiently perform the indicated function. Instead, the negative indices show a potential of the intestinal microbiota to contribute to the establishment or consolidation of important groups of local or systemic diseases. However, the high values of the indices that are negative by themselves do not represent a diagnosis for certain pathologies because they are obtained when the intestinal microbiota has characteristics and that, in the presence of other predisposing conditions (genetics, environment, comorbidities, lifestyle, and food habits), could represent a further predisposing factor towards the group of pathologies indicated [43][47].

3. Microbiota, Dysbiosis Disease, and Personalized Management

After an evaluation of the condition of the microbiota as mentioned above, it can be managed the patient or the person in a more specific way. With this type of detection of a person’s microbiota it could be characterized his dysbiosis and intervene with a targeted therapeutic plan. In fact we must have in mind that the dysbiosis can be: (a) deficiency, resulting from a deficit of the bacterial communities of the intestinal microbiota (*Bifidobacteria spp.* and *Lactobacillus spp.*), mostly favored by a diet poor in soluble fiber and/or rich in packaged, refined, sterilized foods, or consequent to treatments with antibiotics, (b) putrefactive, which is favored by a diet excessively rich in animal fats and meats, and low in fiber with an increase in bacterial populations of *Bacteroides spp*., *Clostridium spp.*, *Peptococcus spp.*, and *Eubacteria spp.*, (c) fermentative, which is characterized by a condition of relative intolerance to carbohydrates or excessive consumption of simple sugars with an increase in bacterial fermentation, (d) sensitization caused by an immune response to components of the normal intestinal microbiota due to deficiency of the immune barrier composed of secretory IgA, and (e) from overgrowing fungi (such as *Candida albicans* and *Saccharomycetes*) favored by a diet rich in simple sugars, leavened foods, refined carbohydrates, and low in fiber [3][4][48]. The various ways to manage and modulate the dysbiotic intestinal microbiota can be

dietary interventions (which also include the use of prebiotics, prebiotics, and postbiotics) and fecal transplantation, to mitigate or treat diseases, such as *C. difficile* infection. Therefore, IBD is one of the best studied conditions associated with dysbiosis; it is heterogeneous with three main subtypes: ulcerative colitis, Crohn's disease, and colitis indeterminate with the microbiome [49][50][51]. These heterogenicities are faced with different therapeutic approaches and therefore the intestinal microbial community present is carefully evaluated. Furthermore, specific diets limiting fermentable oligosaccharides, di-mono-saccharides, and polyols have shown to be beneficial in patients with IBS. In an obese or overweight person, in a metabolic syndrome, or in a patient with type II diabetes, nutritional plans aimed at controlling body weight and restoring the host's energy metabolism, such as the glycemia balance, can be integrated [52][53].

In cardiovascular diseases and cholesterol metabolism, nutritional plans can be integrated (also with probiotics such as Lactobacillus acidophilus and/or Bifidobacterium Bifidum) and changes to lifestyle introduced (tobacco abuse, consumption of alcohol, and others) to help control them; for example, trimethylamine oxidase (TMAO) in atherosclerosis and the inhibition of the microbial enzymes trimethylamine lyases (CutC/D and CntA/B) generating trimethylamine (TMA) from various dietary TMA-containing nutrients. The two TMA lyases have been show to restrict substrate specificity for cleaving choline and carnitine, respectively [51][52][54]. The inhibition of TMA lyases can occur by 3, 3-dimethyl-1-butanol (a structural analog of choline) decreasing bacterial TMA production in a high-choline diet-fed murine model and can be found in olive oil, red wine, and other foods [51][52][55]. Finally, the beneficial effects of food interventions with probiotics with a dysbiotic microbiota on anxiety disorders are further evidence of the involvement and influence of the microbiota and on their appearance. Probiotics, such as *L. rhamnosus*, reduced the anxiety of people who exhibited depressive behaviors. The *B. longum* probiotic has a similar effect, while consuming probiotic milk for 3 weeks significantly improved the psychological situation of the people who received it. These probiotic bacterial strains with specific action in affecting the gut–brain axis can be called "psychobiotics". Furthermore, high doses of prebiotics like trans-galactooligosaccharide (GOS) had a beneficial effect on people with depression [56][57][58].

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